APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1983, p. 31–42
0099-2240/83/010031-12$02.00/0

Vol. 45, No. 1

Effect of Effluent from a Nitrogen Fertilizer Factory and a Pulp Mill on the Distribution and Abundance of *Aeromonas hydrophila* in Albemarle Sound, North Carolina

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Received 27 April 1982/Accepted 8 September 1982

The density of *Aeromonas hydrophila*, standard count bacteria, fecal coliform bacteria, and 18 physical and chemical parameters were measured simultaneously at six sites for 12 months in Albemarle Sound, N.C. One site was above and two sites were below the discharge plume of a Kraft pulping process paper mill. The fourth site was above and the remaining two sites were below the discharge point of a nitrogen fertilizer factory. The impact of the pulp mill on water quality was acute, whereas that of the nitrogen fertilizer factory was chronic and much more subtle. Diffusion chamber studies indicated that *A. hydrophila* survival is increased by pulp mill effluent and decreased by nitrogen fertilizer factory effluent. From correlation and regression analysis, *A. hydrophila* was found to be directly affected by phytoplankton density and, thus, indirectly by concentrations of phosphate, nitrate, and total organic carbon. These two point sources are suspect as indirect causes of red-sore disease epizootics, a disease of fish caused by *A. hydrophila*.

High densities of *Aeromonas hydrophila* have been reported from every basic type of aquatic habitat in the United States (20) and in many other countries (15). Indeed, densities were similar, at the times sampled, for pristine, alkaline lakes in the Grand Tetons (Wyoming), bayous of Louisiana, rain forest streams in Puerto Rico, and off the southwest coast of Puerto Rico in the aphotic zone of the Atlantic Ocean at a depth of 1,000 m (17, 19, 20; Hazen, unpublished data).

It is probably not surprising that a bacterium which can thrive in such a wide variety of aquatic habitats, as *A. hydrophila* does, is also reasonably successful as a facultative pathogen. *A. hydrophila* is known to cause disease in amphibians (8), lizards (23), alligators (14), fish (21, 30), snails (25), cattle (34), and humans (6, 33). At one time, *A. hydrophila* was thought to cause disease only in debilitated humans; however, recently a number of studies have demonstrated that *A. hydrophila* can infect wounds that are exposed during aquatic activity (29).

The positive relationship between numbers of *A. hydrophila* in the water column and prevalence of fish disease (red-sore) has been well documented (10, 16, 18). Miller and Chapman (26) reported the mortality of 37,500 fish in a North Carolina reservoir over one 13-day period due to an epizootic caused by *A. hydrophila*. Such reports are becoming more common in the southeastern United States. Other studies by our laboratory have shown a distinct relationship between water quality and the distribution and abundance of *A. hydrophila* in southeastern reservoirs (16, 18; G. W. Esch and T. C. Hazen, report no. N-153 of the Water Resources Institute of the University of North Carolina, Raleigh). A model has also been developed and used to accurately predict the density of *A. hydrophila* in other reservoirs not used to develop the model (T. C. Hazen, Microb. Ecol., in press).

Albemarle Sound, N.C. has been subject to increased eutrophication recently due to increased population pressure as well as industrial and agricultural operations. The shallow nature of the Sound allows rapid build up of limiting nutrients during dry periods. Thus, algal blooms (*Anabaena* and *Microcystis*) have become common during the late summer and fall (32). Associated with some of these blooms were epizootic outbreaks of red-sore disease. For example, in the fall of 1976, 95% of the white perch population was killed in Albemarle Sound and approximately 50% of the commercial catch of all fish species was discarded because of the presence of unsightly surface lesions associated with red-sore disease (G. Cook, North Carolina Department of Natural Resources and Community Development, personal communication).

The present study was designed to determine whether *A. hydrophila* densities in Albemarle

FIG. 1. Map showing location of study sites in Albemarle Sound, N.C.

Sound could be effected by industrial waste effluents. Indeed, it was hoped that by observing the in situ population dynamics of *A. hydrophila* in disturbed areas of the Sound, a cause-effect relationship with red-sore disease epizootics could be established.

MATERIALS AND METHODS

Study site. The primary area of study was Albemarle Sound (76°N, 36°10'W), located in the northeast corner of North Carolina (Fig. 1). Albemarle Sound is a natural estuary with a mean depth of 3 m, a maximum depth of 20 m, and a shoreline length of 600 km. The total watershed covers 45,695 km^2. Albemarle Sound has two major tributaries, accounting for 83% of the total watershed, the Roanoke River (25,123 km^2) and the Chowan River (12,872 km^2). The nearest connection to the Atlantic Ocean is Oregon Inlet close to Roanoke Island, N.C. The annual mean tidal range at Oregon Inlet is 0.6 m, whereas tides in Albemarle

TABLE 1. Water quality characteristics upstream (site 21) and downstream (sites 29 and 30) from a nitrogen fertilizer factory during July and January 1979[a]

Site	Month	Temp	Cond	DO	pH	Redox	Turb	CAT	CAC
21	January	6.5	105	10.4	6.5	540	90	5	5
	July	28.0	80	7.6	6.3	435	30	48	36
29	January	6.5	110	12.5	6.5	570	90	5	5
	July	28.0	80	8.1	6.5	445	44	47	33
20	January	6.0	170	12.8	7.2	380	57	16	14
	July	27.5	80	8.0	7.8	290	30	59	56

[a] Abbreviations and units: Temp, temperature (degrees Celsius); Cond, conductivity (umho per centimeter); DO, dissolved oxygen (milligrams per liter); Redox, redox potential (mv); Turb, turbidity (Jackson turbidity units); CAT, chlorophyll *A* trichomatic (milligrams per liter); CAC, chlorophyll *A* corrected (milligrams per liter); PA, pheophytin *A* (milligrams per liter); TKN, total Kjeldahl nitrogen (milligrams per liter); NO_{3+2}, nitrate plus nitrite (milligrams per liter); P_i (milligrams per liter); TP, total phosphorus (milligrams per liter); TOC, total organic carbon (milligrams per liter); Hg, mercury (milligrams per liter); SO_4, sulfate (milligrams per liter); SO_2, sulfide (milligrams per liter); NH_4, ammonia (milligrams per liter).

Sound are less than 0.3 m. The characteristic diurnal cycle of tides is approximately 24.8 h. The average annual rainfall in the area is 114 cm. The entire basin supports a mostly rural economy of 500,000 (estimated from the 1970 census). In 1972, commercial fishing was estimated to be producing $5,000,000 annually (2, 5).

A Kraft pulping process paper mill located at Plymouth, N.C. on the Roanoke River, discharges 1.47×10^8 liters day^{-1} into Welch Creek, a small feeder stream that joins the Roanoke River. The mouth of Welch Creek is 9.7 km from the point where the Roanoke River joins Albemarle Sound. Before being pumped into Welch Creek, the pulp mill effluent is aerated while being held in holding ponds for 21 days.

The other point source studied was a nitrogen fertilizer factory near Winton, N.C., on the lower Chowan River. During the course of the present study, this factory was releasing 544 kg of total nitrogen each day into the Chowan River (R. Holman, North Carolina Department of Natural Resources and Community Development, personal communication).

Sampling procedures. Water samples were collected monthly for 1 year in a 2-liter vertical lucite Kemmerer sampling bottle (Wildlife Supply Co., Saginaw, Mich.). The bottle was washed with 70% ethanol after each sample was taken. Each water sample was placed in a sterile 180-ml Whirl Pak bag (NASCO, Ft. Wilkinson, Wis.) and kept on ice for transport to the laboratory; the time from collection to the laboratory never exceeded 1 h.

Bacteriological methods. Densities of *A. hydrophila* were estimated with Rimler-Shotts (R-S) medium (31). A minimum of 1 ml was filtered through a 0.45-μm, gridded, 47-mm-diameter, HA membrane filter (Millipore Corp., Bedford, Mass.). Dilutions were made with filter-sterilized sample water. The number of colony-forming-units (CFU) per ml was recorded as described by Hazen (16) and Hazen et al. (20).

Samples were also analyzed for fecal coliform bacteria with m-FC medium (Difco Laboratories, Detroit, Mich.). Dilutions were made when necessary as above, and samples were filtered through 0.7-μm, gridded, 47-mm-diameter, HC membrane filters (Millipore). CFU were estimated by counting blue colonies after 24 h of incubation at 44.5°C (1).

Standard bacteria counts were determined with TGE medium (Difco). Again, dilutions were made with filter-sterilized sample water and filtered through 0.45-μm, gridded, 47-mm-diameter, HA membrane filters. All colonies were counted after incubation at 35°C for 24 h (1). Bacteria were randomly checked and identified by using the API-20E strip (Analytab Products, Plainview, N.Y.), oxidase, O/129, and fluorescent antibody or serology (7, 12, 16).

Water quality. Five water quality parameters were measured simultaneously with *A. hydrophila* density. Dissolved oxygen, pH, conductivity, temperature, and redox potential were monitored with a Hydrolab Surveyor model 5901 (Hydrolab Corp., Austin, Tex.). Four liters of water was collected and divided into various bottles, and small amounts of the following preservatives were added: nitric acid, sulfuric acid, zinc acetate, and mercuric chloride. All samples were then placed on ice for transport to the laboratory. The appropriately preserved samples were analyzed for the following parameters: ammonia, total Kjeldahl nitrogen, nitrates plus nitrites, sulfates, P_i, total phosphorus, mercury, total organic carbon, sulfides, chlorophyll *A* trichromatic, chlorophyll *A* corrected, and pheophytin *A*. American Public Health Association standard methods (1) were used for all determinations; for details see Esch and Hazen (report no. N-153 of the Water Resources Institute of the University of North Carolina).

Diffusion chamber studies. Pure cultures of *A. hydrophila* (isolated from an infected fish on the Chowan River) were grown in nutrient broth at 35°C for 24 h. Cells were harvested by centrifugation and washed in filter-sterilized, phosphate-buffered saline (pH 7). The number of cells per milliliter was determined with a model ZF Coulter Counter (Coulter Electronics, Hialeah, Fla.) and adjusted to 10^8 cells ml^{-1}.

The final bacteria suspension was then placed into a sterile diffusion chamber just before immersion at the study site. The chambers used had a capacity of 100 ml and a total diffusion surface area of 16,515 mm^2; they were a modification of the MSU-DME chamber (24). O-rings were added to each chamber to reduce contamination and leakage. A 0.45-μm, 142-mm-diameter,

TABLE 1—*Continued*

PA	TKN	NO_{3+2}	P_i	TP	TOC	Hg	SO_4	SO_2	NH_4
5	0.4	0.06	0.06	0.11	18	0.2	24	0.1	0.02
19	0.6	0.15	0.02	0.11	10	0.2	8	0.1	0.02
5	0.6	0.19	0.02	0.06	17	0.2	15	0.1	0.13
23	0.9	0.11	0.02	0.13	12	0.2	9	0.1	0.07
5	0.5	0.1	0.02	0.02	19	0.2	9	0.1	0.02
5	0.8	0.02	0.02	0.08	14	0.9	8	0.1	0.02

TABLE 2. Water quality characteristics upstream (site 31) and downstream (sites 30 and 28) from a pulp mill during July and January 1979[a]

Site	Month	Temp	Cond	DO	pH	Redox	Turb	CAT	CAC
31	January	6.0	110	11.0	6.5	400	16	5	15
	July	26.0	240	2.2	6.1	340	30	20	11
30	January	7.5	950	9.0	7.5	350	104	15	16
	July	30.0	1,800	0.4	7.3	195	258	25	5
28	January	6.5	120	11.0	7.0	390	42	14	12
	July	28.0	110	7.1	6.5	365	4	34	17

[a] For abbreviations and units, see footnote *a* of Table 1.

nylon-reinforced, Acropor membrane filter (Gelman Instrument Co., Ann Arbor, Mich.) was used to create the diffusion surface.

In June 1979 five chambers were suspended 1 m below the surface at site 21, above the nitrogen fertilizer factory (Fig. 1). Another five chambers were suspended 500 m downstream from the factory at site 29. Samples of 1 ml were taken from each chamber with a sterile syringe at regular intervals for 114 h. Each sample was immediately fixed in 10% phosphate-buffered Formalin (pH 7) and refrigerated for later reading with the Coulter Counter. This technique preserves Coulter Counter counts for more than 2 weeks (Hazen, unpublished data).

In July 1979 five chambers were suspended 1 m below the surface at site 31, 1 km upstream from the effluent point source of the Kraft pulping process paper mill (Fig. 1). Another five chambers were suspended 1 m below the surface at site 30, the pulp mill effluent point source. Samples were again taken, as above, at regular intervals for 108 h. During both chamber studies, water quality was monitored as stated above simultaneously with each sample collection.

Data analysis. Programs developed on an IBM 370-148 computer were used for all statistical tests. Two-factor analysis of variance was used to test differences between sites and times. Multiple correlation and regression were used to determine relationships between densities of *A. hydrophila* and water quality parameters. Heteroscedastic data as determined by skew and kurtosis were made more homoscedastic by transformation with $\log(x + 1)$. Any statistical probability greater than or equal to 0.05 was considered significant (35).

RESULTS

Water quality. When compared with the upstream station (site 21), the two sampling sta-

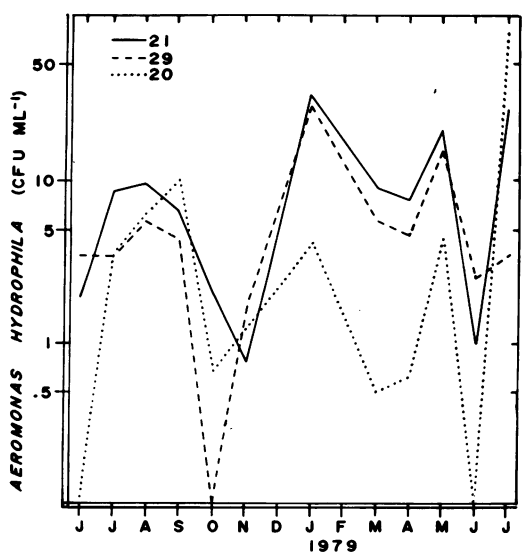

FIG. 2. Densities of *A. hydrophila* by months at three sites associated with a nitrogen fertilizer factory effluent (sites 29 and 20 downstream and site 21 upstream).

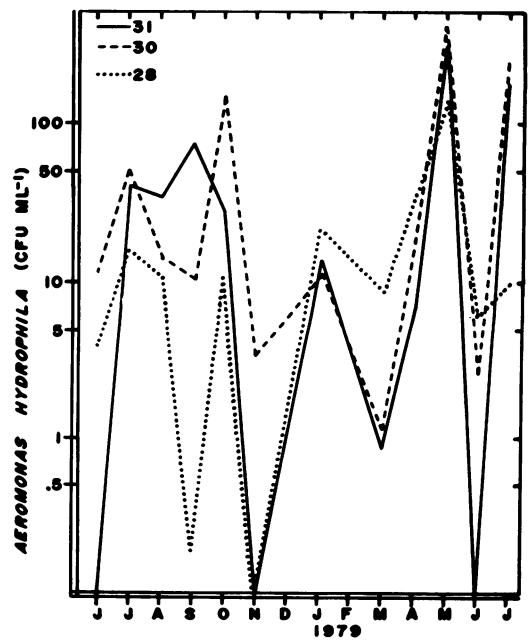

FIG. 3. Densities of *A. hydrophila* by months at three sites associated with a pulp mill effluent (sites 30 and 28 downstream and site 31 upstream).

TABLE 2—*Continued*

PA	TKN	NO_{3+2}	P_i	TP	TOC	Hg	SO_4	SO_2	NH_4
5	0.7	0.02	0.02	0.13	15	0.2	17	0.1	0.12
17	0.9	0.08	0.02	0.02	25	0.2	19	0.1	0.09
5	2.4	0.02	0.2	0.39	160	0.6	100	0.1	0.66
14	7.2	0.02	0.42	0.84	290	0.2	140	0.1	3.20
5	0.4	0.31	0.02	0.02	5	0.2	13	0.1	0.02
13	0.3	0.05	0.02	0.02	20	0.2	11	0.1	0.02

tions downstream from the nitrogen fertilizer factory (sites 29 and 20) had significantly higher dissolved oxygen, pH, chlorophyll *A* trichromatic, chlorophyll *A* corrected, total phosphorus, ammonia, nitrate plus nitrite, and conductivity. The redox potential, however, was significantly lower at site 21. Monthly differences were significant for the following parameters: temperature, dissolved oxygen, redox potential, turbidity, total phosphorus, total organic carbon, sulfate, ammonia, total Kjeldahl nitrogen, and nitrate plus nitrite. A representative data set for the three sites during January and July is shown in Table 1. The sites downstream from the pulp mill (sites 30 and 28), when compared to the upstream site (site 31), had significantly higher conductivity, pH, turbidity, pheophytin *A*, total Kjeldahl nitrogen, nitrate plus nitrite, P_i, total phosphorus, total organic carbon, sulfate, and ammonia. The following parameters were significantly lower by site: dissolved oxygen and redox potential. Monthly differences were significant for the following parameters: temperature, conductivity, dissolved oxygen, turbidity, chlorophyll *A* corrected, and pheophytin *A*. A representative data set for these parameters at these sites during January and July is shown in Table 2. All differences in water quality by sites and months were determined by factorial analysis of variance.

Bacteria distribution and abundance. Factorial analysis of variance indicated significant differences in *A. hydrophila* densities by month at the nitrogen fertilizer factory ($F = 2.3$, df = 11 and 35, $P < 0.05$), but not by site (Fig. 2). At the pulp mill, densities of *A. hydrophila* were significantly different by month ($F = 5.4$, df = 11 and 35, $P < 0.001$) and site ($F = 3.9$, df = 2 and 35, $P < 0.05$). Figure 3 shows that site 30, the pulp mill

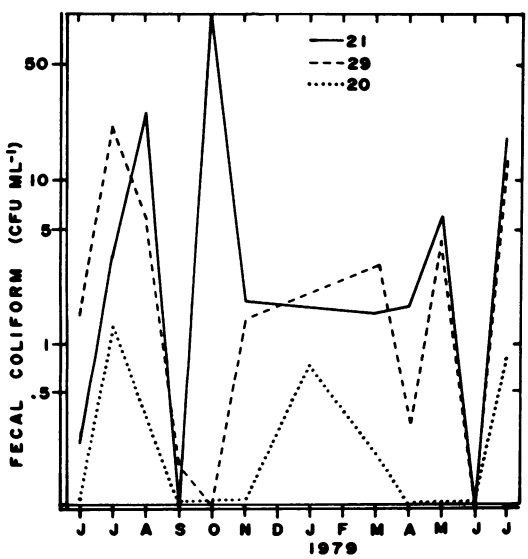

FIG. 4. Densities of fecal coliform bacteria by month at three sites associated with a nitrogen fertilizer factory effluent (sites 29 and 20 downstream and 21 upstream).

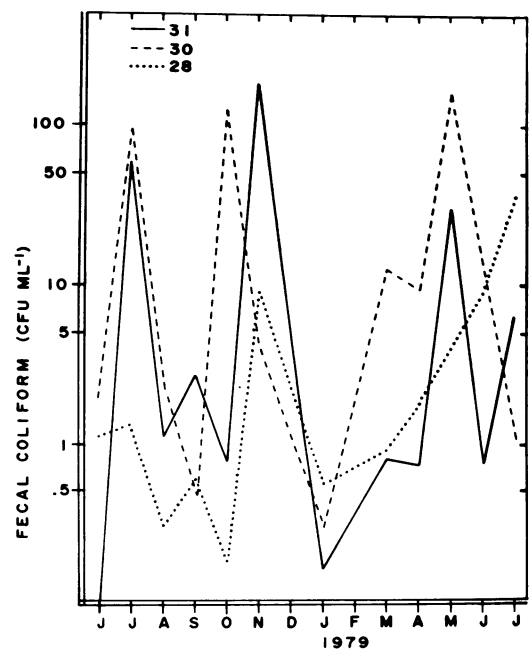

FIG. 5. Densities of fecal coliform bacteria by month at three sites associated with a pulp mill effluent (sites 30 and 28 downstream and site 31 upstream).

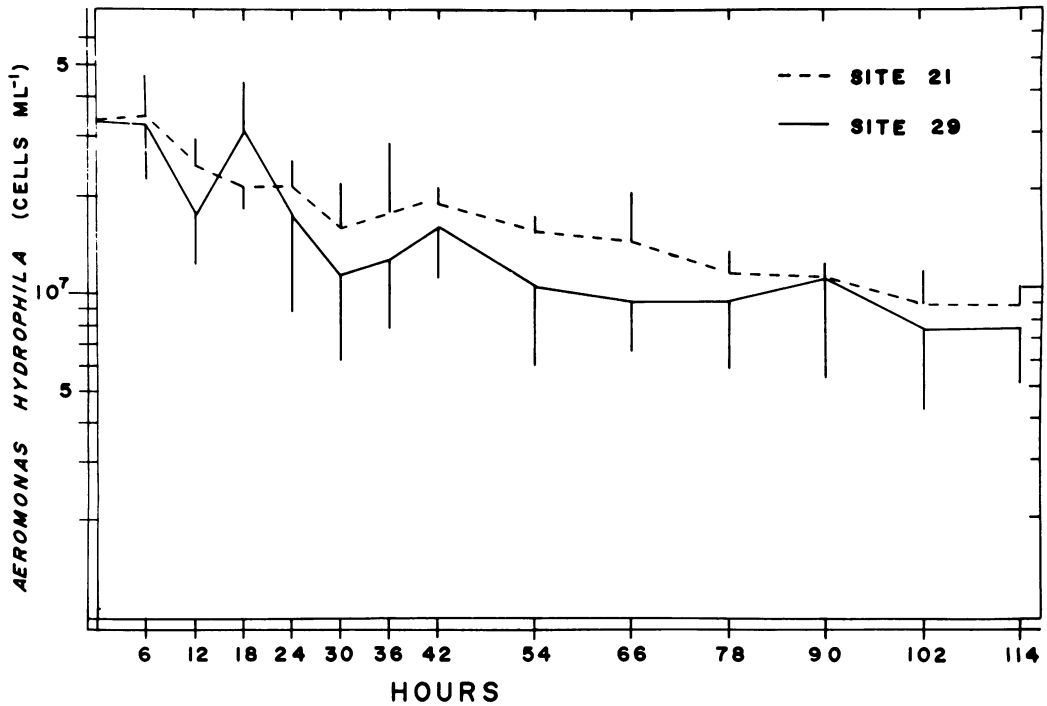

FIG. 6. Survival in situ of *A. hydrophila* at a nitrogen fertilizer factory effluent (site 29) and upstream (site 21).

effluent point source, had very significantly higher densities of *A. hydrophila* than the upstream or downstream sites (sites 31 and 28, respectively). Differences by month followed patterns previously observed for this bacteria, with a spring maximum and a brief fall resurgence at both sites (Fig. 2 and 3). The highest densities (300 CFU ml^{-1}) occurred in the pulp mill effluent (site 30).

Standard count bacterial densities at the nitrogen fertilizer factory were not significantly different by month or by site. However, factorial analysis of variance revealed significant differences for standard count bacteria for sites (F = 4.6, df = 2 and 35, $P < 0.05$) and months (F = 3.9, df = 11 and 35, $P < 0.002$) within the pulp mill effluent. Site 30, the outfall, had the highest densities ($>10^8$ CFU ml^{-1}). Both sampling locations showed a fall maximum; however, variation was great during all seasons.

Densities of fecal coliform bacteria, when analyzed by factorial analysis of variance, were found to be significantly different by site (F = 5.7, df = 2 and 35, $P < 0.02$), but not by month for the nitrogen fertilizer factory sites (Fig. 4). The pulp mill location had significantly higher densities at the outfall site 30; however, large variations in densities, because of back flushing at the upstream site 31 made overall site differences between the three sites nonsignificant (Fig. 5). In addition, there was no significant difference by month in densities of fecal coliform bacteria at the pulp mill location. The highest densities of fecal coliforms were recorded at site 30, the pulp mill outfall (150 CFU ml^{-1}).

Survival of *A. hydrophila*. The densities of *A. hydrophila* in diffusion chambers at sites 21 and 29 declined slowly, but significantly (F = 7.75, df = 1 and 84, $P < 0.02$), over the 114-h sampling period (Fig. 6). Differences between the two sites became significant after 42 h. At site 29, the one closest to the nitrogen fertilizer factory, survival of *A. hydrophila* was significantly lower.

The density of *A. hydrophila* in diffusion chambers at the pulp mill sites (sites 30 and 31) also declined significantly (F = 373.9, df = 1 and 72, $P < 0.0001$) and steadily over time (Fig. 7). However, site 31 had significantly lower densities of *A. hydrophila* after only 24 h. In addition, densities of *A. hydrophila* in chambers at site 31 continued to decline until the study was terminated at 108 h when the difference in chamber densities at the two sites was greater than 1 order of magnitude.

Over the time course of the study at sites 21 and 29, total Kjeldahl nitrogen and nitrate plus nitrite concentration were significantly higher at site 21. Ammonia concentration was higher at site 29, but not significantly. None of the other water quality parameters was significantly different.

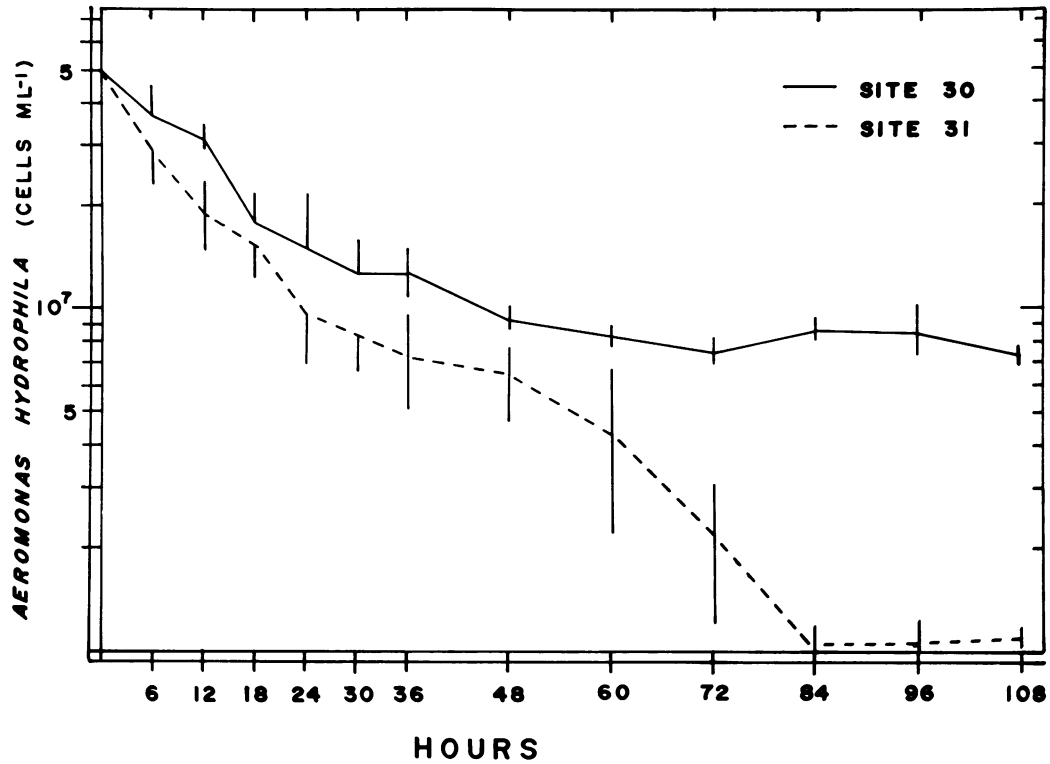

FIG. 7. Survival in situ of *A. hydrophila* at a pulp mill effluent (site 30) and upstream (site 31).

During the diffusion chamber study at the pulp mill sites (sites 30 and 31), pheophytin *A*, turbidity, ammonia, total Kjeldahl nitrogen, P_i, total phosphorus, conductivity, and pH were all significantly higher at site 30, the effluent outfall, than at other stations. Redox potential, dissolved oxygen, and nitrate plus nitrite were all significantly lower at site 30. The other water quality parameters were not significantly different.

Water quality and densities of *A. hydrophila*. A best-fit regression for the nitrogen fertilizer factory sites showed that total organic carbon, chlorophyll *A* trichromatic, standard count bacteria, pheophytin *A*, turbidity, total phosphorus, and conductivity could account for 75.2% of the variation in densities of *A. hydrophila* (Tables 3 and 4). All water quality parameters significantly affected the slope, giving a highly significant regression. Total organic carbon, pheophytin *A*, and conductivity negatively affected the slope, whereas all the other parameters had a positive effect.

The pulp mill sites yielded a best-fit regression, with chlorophyll *A* trichromatic and chlorophyll *A* corrected accounting for 23% of the variation in densities of *A. hydrophila* (Tables 5 and 6). None of the other parameters, in combination, significantly affected the slope of the regression; the regression was significant.

DISCUSSION

The nitrogen fertilizer factory on the lower Chowan River significantly increased nitrogen input to Albemarle Sound (544 kg of total nitrogen per day). As reported by Stanley and Hobbie (32), this accounts for 10% of the total nitrogen input for the Chowan River basin. Consequently, blooms of phytoplankton are common in the lower Chowan River and Albemarle Sound throughout summer and fall. This was also supported in the present study by significantly higher chlorophyll *A* concentrations at downstream stations. Stanley and Hobbie (32) indicated that these blooms consist mainly of *Anabaena, Microcystis*, and *Aphanozomenon* during the summer and diatoms (*Melosira*) during the winter. These phytoplankton blooms are probably the primary cause of the significantly higher dissolved oxygen, total phosphorus, and conductivity and significantly lower redox potential at sampling stations downstream from the nitrogen fertilizer factory effluent source. Heavy rainfall during the spring and early summer has a flushing affect on the system, since no differences are observed at these

TABLE 3. Correlation matrix for water quality parameters at sites associated with the nitrogen fertilizer factor (sites 21, 29, and 20)[a]

	Aero	Fecal	SCB	Temp	Cond	DO	pH	Redox	Turb
Aero	1.000								
Fecal	0.159	1.000							
SCB	**0.378**	−0.200	1.000						
Temp	0.282	0.174	−0.245	1.000					
Cond	−0.199	−0.106	**0.552**	**−0.647**	1.000				
DO	−0.233	−0.240	0.125	0.051	0.349	1.000			
pH	0.101	**−0.357**	0.328	−0.033	**0.491**	**0.580**	1.000		
Redox	−0.166	0.271	−0.185	−0.283	−0.128	**−0.461**	**−0.715**	1.000	
Turb	−0.056	−0.017	0.034	**−0.377**	0.290	**0.429**	−0.160	−0.158	1.000
CAT	0.259	−0.193	0.127	**0.431**	0.071	**0.389**	**0.649**	**−0.422**	−0.207
CAC	0.308	−0.227	0.172	**0.356**	0.088	0.311	**0.654**	**−0.399**	−0.269
PA	−0.076	0.068	−0.125	**0.478**	−0.173	0.313	0.009	−0.081	0.103
TKN	**0.422**	0.058	0.266	0.353	−0.127	−0.201	0.055	−0.022	**−0.400**
NO_{3+2}	−0.018	0.114	−0.065	−0.112	−0.247	−0.264	**−0.553**	**−0.531**	−0.199
PO_4	−0.131	−0.011	0.252	−0.339	**0.472**	−0.078	0.005	0.136	0.226
TP	0.122	**0.370**	−0.243	**0.482**	**−0.490**	−0.314	**−0.569**	**0.396**	−0.211
TOC	0.041	−0.099	−0.180	0.354	**−0.614**	−0.063	**−0.420**	0.116	−0.256
HG	0.184	−0.172	**−0.455**	0.073	0.227	0.306	−0.025	0.033	0.270
SO_4	−0.070	0.124	0.157	−0.059	0.266	0.337	0.025	−0.053	0.179
SO_2	0.001	0.001	0.005	0.003	0.006	0.005	0.011	0.011	0.009
NH_4	−0.102	0.152	0.034	−0.226	0.144	−0.102	−0.231	0.244	0.145

[a] Aero, *A. hydrophila*; Fecal, fecal coliform bacteria; SCB, standard count bacteria. All other abbreviations are as described in footnote *a* of Table 1. $n = 30$; $P < 0.05$ when $r \geq 0.355$ (indicated by boldface).

times for any water quality parameter. However, during drier periods in the summer and fall, nutrient buildup is rapid, due to the shallow nature and slower flushing rates of Albemarle Sound and the Chowan River. As indicated by the present study and that of Stanley and Hobbie (32), effluent from the fertilizer plant is an important source of eutrophication for Albemarle Sound.

The pulp mill effluent is, almost literally, a nutrient soup. It significantly increases turbidity, conductivity, pH, pheophytin *A*, total nitrogen, nitrate plus nitrite, P_i, total phosphorus, total organic carbon, sulfate, and ammonia at downstream stations, and it significantly lowers dissolved oxygen and redox potential. Most of these affects are direct, although some, such as lowered dissolved oxygen, are indirect, being produced as a result of increased bacterial respiration. Increased conductivity and pH are the result of chemicals (calcium bisulfite, sodium hydroxide) added during the Kraft paper processing, but which remain in the final effluent released from holding ponds. Welch Creek is significantly affected throughout the year from the point source to its junction with the Roanoke River. The affect on the Roanoke River varies with rainfall received within the basin; the maximum influence on the river and Albemarle Sound occurs during the drier summer and fall months.

Bacteria distribution and abundance. The highest densities of *A. hydrophila*, standard count bacteria, and fecal coliform bacteria were recorded at the pulp mill effluent site. All were significantly higher at the outfall and the downstream sites than upstream. Although all three bacteria parameters were higher at the nitrogen fertilizer factory point source, none was significantly different from those at the downstream sites. The range of densities of *A. hydrophila* observed at the various sites (0.1 to 100 CFU ml^{-1}) was within the range of values previously reported for unpolluted lakes, rivers, estuaries, and reservoirs in various areas in the United States (20). However, *A. hydrophila* densities in the effluent waters of the pulp mill and the nitrogen fertilizer factory were, in general, lower when compared with other polluted freshwaters (20).

Densities of fecal coliform bacteria exceeded recreational water standards (10 CFU ml^{-1}) at the nitrogen fertilizer factory sites during 3 months of the 12-month study and exceeded drinking water standards (0.1 CFU ml^{-1}) at all sites for all months (3). The pulp mill effluent sites exceeded recreational water standards for coliform densities during 8 months and drinking water standards for all months sampled. Recent studies (4, 22) have shown that as much as 80% of fecal coliform isolates from pulp mill effluents may be *Klebsiella pneumoniae*, another potential pathogen of humans and other animals; the combination of *K. pneumoniae* and *A. hydrophila* probably accounts for more than 90% of all fecal coliform-positive bacteria in pulp mill ef-

TABLE 3—*Continued*

CAT	CAC	PA	TKN	NO_{3+2}	PO_4	TP	TOC	HG	SO_4	SO_2	NH_4
1.000											
0.976	1.000										
0.386	0.200	1.000									
0.517	**0.553**	0.207	1.000								
−0.375	**−0.395**	0.168	0.099	1.000							
−0.151	−0.119	−0.124	−0.192	−0.136	1.000						
−0.050	−0.139	**0.541**	0.267	**0.454**	0.152	1.000					
−0.054	−0.096	0.240	0.148	**0.516**	−0.278	**0.486**	1.000				
−0.045	−0.045	0.129	0.149	0.160	−0.075	−0.009	0.001	1.000			
−0.168	−0.168	−0.076	−0.074	0.001	−0.265	−0.123	−0.221	0.176	1.000		
0.001	0.001	0.001	0.004	0.003	0.006	0.006	0.011	0.002	0.002	1.000	
−0.224	−0.213	0.006	0.333	**0.370**	−0.094	0.162	−0.027	**0.439**	−0.007	0.002	1.000

fluents. The levels of fecal coliforms observed in Welch Creek (sites 30 and 31) indicate that this system is unsuitable for recreational use. Similar levels of contamination have also been demonstrated for other, untreated, pulp mill effluents (4, 22).

Densities of *A. hydrophila* followed a seasonal pattern at all sites, similar to that observed previously in the southeastern United States; spring maxima were followed by a decline during the summer and then by a fall resurgence (16; Hazen, in press). This pattern of seasonality parallels seasonal changes in phytoplankton densities, with peak densities in *A. hydrophila* coinciding with algal blooms. The association between *A. hydrophila* and these primary producers is further supported by the strong positive correlations of densities of *A. hydrophila* and chlorophyll *A* concentration.

Fecal coliform bacteria did not show a distinct seasonality at either location. Densities of fecal coliform bacteria were lower during algal blooms as demonstrated by the negative correlation between densities of fecal coliform bacteria and chlorophyll *A* concentrations. In addition, fecal coliform densities were significantly correlated with densities of *A. hydrophila* at the pulp mill sites, but not at the nitrogen fertilizer factory sites.

Survival of *A. hydrophila* and water quality. *A.*

TABLE 4. Best-fit regression statistics for *A. hydrophila* at sites associated with the nitrogen fertilizer factory (sites 21, 29, and 30)[a]

Determination	Source or variable	Sum of squares	Degrees of freedom	Mean square	*F* statistic	*B* coefficient	SE of *B*	*T* statistic[b]
Analysis of variance	Regression	11.387	7	1.627	9.51[c]			
	Residuals	3.763	22	.171				
	Total	15.152	29					
Analysis of coefficients	TOC					−2.1033	0.5282	−3.982
	CAT					0.0204	0.0047	4.318
	SCB					0.5129	0.0839	6.115
	PA					−0.0757	0.0193	−3.916
	Turb					0.0530	0.0124	4.268
	TP					13.7954	4.0727	3.387
	Cond					−0.0392	0.0068	−5.783

[a] All abbreviations are as in footnote *a* of Table 1 and footnote *a* of Table 3. The correlation coefficient (r) was 0.8670 ($r^2 = 0.3223$). When adjusted to account for the biased estimates of the population parameter, $r = 0.7516$ ($r^2 = 0.3104$); $n = 30$, y intercept $= -1.1803$.

[b] $P < 0.05$ when $T \geq 2.074$.

[c] $P < 0.0001$.

TABLE 5. Correlation matrix for water quality parameters at sites associated with the pulp mill (sites 30, 31, and 28)[a]

	Aero	Fecal	SCB	Temp	Cond	DO	pH	Redox	Turb
Aero	1.000								
Fecal	0.341	1.000							
SCB	−0.155	0.072	1.000						
Temp	0.028	−0.272	**−0.423**	1.000					
Cond	0.143	0.052	0.136	0.289	1.000				
DO	−0.133	0.009	−0.269	−0.200	**−0.664**	1.000			
pH	0.042	0.074	0.328	−0.250	**0.518**	−0.219	1.000		
Redox	0.042	−0.026	**−0.364**	0.019	**−0.688**	**0.655**	**−0.559**	1.000	
Turb	0.033	0.062	−0.012	−0.317	**−0.703**	**0.703**	**−0.445**	**0.644**	1.000
CAT	−0.191	−0.257	−0.150	**0.471**	0.300	−0.312	−0.004	**−0.531**	−0.256
CAC	−0.287	−0.197	−0.109	**0.399**	0.204	−0.279	−0.071	**−0.465**	−0.161
PA	0.009	0.014	−0.056	0.267	**0.564**	**−0.468**	0.292	**−0.633**	**−0.547**
TKN	**0.370**	0.169	0.229	0.185	**0.745**	**−0.640**	**0.469**	**−0.639**	**−0.810**
NO_{3+2}	−0.045	−0.196	−0.245	−0.201	−0.201	**0.451**	−0.003	0.259	**0.380**
PO_4	−0.183	0.113	**0.563**	0.135	**0.459**	**−0.493**	**0.401**	**−0.590**	**−0.505**
TP	0.117	−0.005	0.314	0.308	**0.817**	**−0.754**	**0.518**	**−0.799**	**−0.902**
TOC	0.116	0.025	0.114	**0.398**	**0.809**	**−0.721**	**0.488**	**−0.778**	**−0.881**
HG	−0.237	−0.206	−0.136	**0.441**	**0.499**	−0.167	0.145	−0.246	−0.146
SO_4	−0.021	−0.073	**0.761**	−0.208	**0.369**	**−0.495**	**−0.430**	**−0.594**	**−0.434**
SO_2	0.001	0.001	0.001	0.003	0.002	0.002	0.009	0.005	0.002
NH_4	0.016	−0.121	0.183	**0.424**	**0.725**	**−0.653**	**0.434**	**−0.736**	**−0.731**

[a] All abbreviations are as in footnote *a* of Table 1 and footnote *a* of Table 3. $n = 30$; $P < 0.05$ when $r \geq 0.355$ (indicated by boldface type).

hydrophila survived best in the pulp mill effluent. The pulp mill effluent sustained a population density of *A. hydrophila* of 10^7 cells ml^{-1}, whereas the upstream site (site 31) supported a density that was more than two orders of magnitude lower. Differences in *A. hydrophila* densities in chambers at the two sites became significant after just 24 h. Compared with site 30, all sites sampled would be considered as relatively nutrient poor, accounting for the increased survival of *A. hydrophila* at this site. Since the strain of *A. hydrophila* employed in the study was isolated from an infected fish from the Chowan River, it is reasonable to assume that the pulp mill effluent may significantly increase the probability of red-sore disease in fish.

At the nitrogen fertilizer factory outfall differences in survivorship of *A. hydrophila* were not significant from the upstream station during the first 48 h. At the termination of the experiment (114 h) both sites had chamber densities close to 10^7 cells ml^{-1}. However, over the entire time course of the study, the survival of *A. hydrophila* at site 29 was significantly less than that upstream. The only water quality parameter which can be related to this difference is ammonia. Moreover, ammonia is also significantly negatively correlated with densities of *A. hydrophila* at both locations. Other studies have also demonstrated a significant negative correlation between densities of *A. hydrophila* and ammonia (17; Hazen, in press; Esch and Hazen, report no. N-153 of the Water Resources Institute of the University of North Carolina). In addition, laboratory studies indicate that low concentrations of ammonia, even under optimal conditions for growth, can significantly increase the generation time of *A. hydrophila* (Rivera and Hazen, unpublished data). The higher densities of *A. hydrophila* observed downstream from the nitrogen fertilizer factory during regular monthly sampling does not agree with the chamber study results. A possible explanation is that closer contact of the bacteria with the phytoplankton, when not in a diffusion chamber, overcomes the toxic effects of the increased ammonia concentration by supplying high-quality nutrients (leaked carbon compounds from the algae).

Previous diffusion chamber studies with *A. hydrophila* in thermally altered reservoirs (11) revealed that elevated temperature had a strong influence on increasing survival of *A. hydrophila*. However, this effect is probably indirect since elevation of water temperature in these reservoirs has also been shown to significantly increase primary production, i.e., phytoplankton density (13).

The best-fit regressions for both locations reveal that chlorophyll *A* concentration has the greatest effect on the density of *A. hydrophila*. Consequently, small increases in P_i and nitrates in this system presumably have a great effect on the density of phytoplankton which, in turn, causes increases in densities of *A. hydrophila*. Intimate interactions between *A. hydrophila* and phytoplankton can, however, reduce to some extent the toxic effects of other water quality factors such as ammonia. The present investiga-

TABLE 5—*Continued*

CAT	CAC	PA	PKN	NO_{3+2}	PO_4	TP	TOC	HG	SO_4	SO_2	NH_4
1.000											
0.957	1.000										
0.677	**0.528**	1.000									
0.173	0.003	**0.601**	1.000								
−0.097	−0.090	−0.074	−0.296	1.000							
0.283	0.305	0.223	**0.433**	**−0.367**	1.000						
0.384	0.274	**0.618**	**0.864**	**−0.398**	**0.730**	1.000					
0.453	0.339	**0.660**	**0.861**	−0.298	**0.587**	**0.941**	1.000				
0.549	**0.598**	0.304	−0.045	0.165	0.249	0.231	0.275	1.000			
0.186	0.144	0.327	**0.455**	−0.230	**0.376**	**0.501**	**0.413**	0.017	1.000		
0.001	0.001	0.001	0.002	0.002	0.002	0.002	0.001	0.002	0.001	1.000	
0.485	**0.401**	**0.519**	**0.755**	−0.258	**0.738**	**0.907**	**0.912**	0.323	0.329	0.001	1.000

tion supports other studies which have also demonstrated correlations between a relative eutrophic index and densities of *A. hydrophila* (28). Their eutrophic index included total phosphorus, dissolved phosphorus, inorganic nitrogen, secchi depth, chlorophyll *A*, and hypolimnetic dissolved oxygen. It is thus becoming more and more apparent that increases in densities of *A. hydrophila* in aquatic systems are intimately linked to increases in primary productivity.

The results of the present study clearly indicate that effluent from the fertilizer factory and the pulp operation are contributing to eutrophication in Albemarle Sound and, hence, the survival and population dynamics of *A. hydrophila*. Other bacteria species may behave similarly, but not necessarily the same as *A. hydrophila*. Effluent from the nitrogen fertilizer factory probably has the greatest effect on increasing eutrophication, whereas the pulp mill effluent has the greatest effect on increasing directly the densities of potential pathogens of fish and humans. The role that *A. hydrophila* plays in natural aquatic systems, in terms of nutrient cycling, etc., is still unknown; however, since *A. hydrophila* can represent from 10 to 75% of the total bacteria population within fresh and marine systems, its role must be considerable (3, 16, 27). Other studies have shown a strong positive correlation between density of *A. hydrophila* in the water column and the prevalence of red-sore disease in fish over a 4-year period in a South Carolina reservoir (9, 16) and in six North Carolina reservoirs (Hazen, in press; 18). It is concluded that reduction of phosphate and nitrogen inputs into Albemarle Sound, and perhaps other systems as well, will not only reduce the eutrophication process, but will also minimize the

TABLE 6. Best-fit regression for *A. hydrophila* at sites associated with the pulp mill (sites 30, 31, and 28)[a]

Determination	Source or variable	Sum of squares	Degrees of freedom	Mean square	*F* statistic	*B* coefficient	SE of *B*	*T* statistic[b]
Analysis of variance	Regression	3.190	2	0.397	4.20[c]			
	Residuals	10.718	27	1.595				
	Total	13.907	29					
Analysis of coefficients	CAT					0.0413	0.0182	2.266
	CAC					−0.0567	0.0213	−2.663

[a] All abbreviations are as in footnote *a* of Table 1 and footnote *a* of Table 3. The correlation coefficient (r) was 0.4789 (r^2 = 0.3427). When adjusted to account for the biased estimates of the population parameter, r = 0.2293 (r^2 = 0.2175); n = 30, y intercept = 1.2579.

[b] $P < 0.05$ when $T \geq 2.052$.

[c] $P < 0.05$.

probability of red-sore epizootics among resident fish species.

ACKNOWLEDGMENTS

We are grateful to William F. Crawford and Edgar Roberts for their outstanding technical assistance in various aspects of this study. We are also grateful to Andy Roberts, James Matthews, Anne Mansfield, Greg Olsen, Mark Mondl, Joe Camp, Cecile Smith, and Gayle Hazen for their technical assistance. Throughout this study we were given extremely generous technical support by Steve Tedder and his associates within the Division of Environmental Management of the North Carolina Department of Natural Resources and Community Development.

This work was supported by grant B-112-NC from the North Carolina Water Resources Research Institute. It was also supported in part by a grant from the North Carolina Board of Science and Technology, by contract DE-AS09-76EY-0900 between Wake Forest University and the Department of Energy, by a grant from the Oficina de Coordinacion de Estudios Graduados y Investigaciones of the University of Puerto Rico, by Public Health Service grant RR-8102 from the National Institutes of Health to the University of Puerto Rico, and by Sea Grant (National Oceanic and Atmospheric Administration, Department of Commerce) UPR SG 04F15844030 project En/P-45.

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